

ACUTE ASCENDING MYELITIS FOLLOWING A MONKEY
BITE, WITH THE ISOLATION OF A VIRUS CAPABLE
OF REPRODUCING THE DISEASE

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PLATES 13 TO 15

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It is well known that acute ascending myelitis is not a single disease; it is a syndrome, which is characterized chiefly by rapidly ascending paralysis and high mortality and which may occur in the course of various forms of acute myelitis. Of the so called infectious myelitides, those which follow a host of various bacterial and virus diseases constitute the more frequent types, and are generally referred to as secondary or postinfectious myelitis. Pathologically this form of myelitis is characterized by perivascular demyelination. Its pathogenesis and etiology are still obscure. Primary infectious myelitis is almost unknown, with the exception of rare cases of the so called spinal form of epidemic encephalitis, of acute anterior poliomyelitis which involves the remainder of the cord, and of certain recently described cases of acute ascending myelitis in which rabies virus is the probable cause (1). The purpose of the present communication is to describe, firstly, a fatal case of acute ascending myelitis which followed the bite of an apparently normal monkey, and, secondly, the isolation from the brain and cord and from the spleen of that case of a filtrable virus which reproduces the disease in rabbits.

History.—Dr. W. B., 29 years old, was engaged in experimental work on poliomyelitis. On Oct. 22, 1932, he was bitten on the dorsum of the left ring and little fingers at the terminal phalangeal joints, by an apparently normal *Macacus rhesus* monkey. The wounds, which were superficial, were painted with iodine and then with alcohol, and Dr. B. continued his work. The monkey died under ether

during an operation; no pathological examination was made. 3 days later, Dr. B. noticed pain, redness, and slight swelling at the sites of the bites. A lymphangitis developed and soon there was enlargement and tenderness of the left epitrochlear and axillary lymph nodes. In the afternoon of Oct. 28, he was admitted to the Third Surgical Service of Bellevue Hospital. His temperature was 101.4°F., the pulse was 90; physical examination revealed only the superficial redness and slight induration over the dorsum of the terminal phalanges of the left little and ring fingers with an associated regional lymphangitis and epitrochlear and axillary lymphadenitis. On the day of admission he received a prophylactic injection of tetanus antitoxin. In the course of the next few days he appeared to improve considerably; several small vesicles, containing a small amount of cloudy fluid, formed at the sites of the bites; the vesicles were opened on Oct. 30. The lymphangitis disappeared; the regional lymph nodes diminished somewhat in size and were only slightly tender.

On Nov. 1, 7 days after the first signs of infection of the fingers appeared, he developed generalized abdominal cramps, which lasted for 2 days and were not associated with tenderness, rigidity, nausea, vomiting, or diarrhea. On Nov. 4, he developed marked hyperesthesia of the lower extremities associated with urinary retention. Physical examination at that time revealed a generalized hyperalgesia below the level of the umbilicus; the knee jerks and abdominal reflexes were absent; the ankle jerks and cremasterics were present. The Babinski sign was negative; there were no signs of meningeal irritation; the upper extremities were not involved. At his own request he was given 20 cc. of convalescent poliomyelitic serum. The following day, Nov. 5, there was flaccid paralysis of both lower extremities. A spinal puncture performed that day yielded a clear fluid under slightly increased pressure with no evidence of block. Microscopic examination showed 112 cells per c. mm., all monocytes, albumen +, globulin +, reducing body 75.9 mg. per 100 cc.; smears and culture of the fluid were negative. On Nov. 5, after a neurological consultation by Drs. F. Kennedy and E. D. Friedman, the latter made the following note: "Pupils and other cranials negative. Upper extremities normal. Paraplegia involving all the muscles from the costal arch downward. Abdominals not obtained. Knee jerks absent; ankle jerks—left greater than right. No Babinski—plantars ventral. There is a level at about D 7 to D 8 below which pain and temperature senses are diminished. Tactile and posterior column sense not seriously altered. Bladder retention. Findings are those of a ventral myelitic lesion at the level noted. The etiology is obscure although the recent infection is probably related to the spinal condition." During the next day, Nov. 6, the sensory level had ascended to D 3, the ankle jerks were still present, and the upper extremities remained normal. On Nov. 7, he complained of paresthesias in the upper extremities and Dr. Friedman made the following note: "Pupils, fundi, and other cranials negative except for a few nystagmoid jerks in horizontal plane. Upper extremities normal; biceps and triceps jerks present. No Horner syndrome. Breathing mechanism intact." By this time

the ulcer on the little finger had entirely healed and was covered by a scab, and the one on the ring finger had become filled with granulation tissue. That night the temperature rose to 104.8°F. The following morning, Nov. 8, the temperature dropped to 99°F., but the patient looked very ill and complained of pain in the upper extremities. During the course of the day, hiccoughing developed and the respirations became slow and irregular. During the evening the respiratory

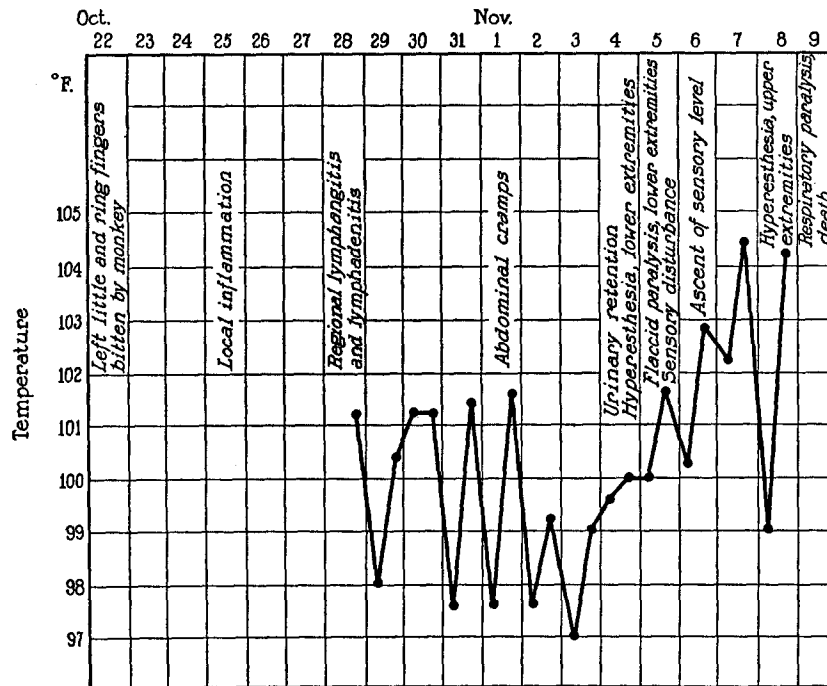


CHART 1. Clinical course of the human disease.

rate diminished to six a minute; he became quite cyanotic, and was put into a respirator. About 75 minutes later he had a convulsion, with apparently laryngeal spasm, and lost consciousness. Pulmonary edema developed, the frothy fluid being pumped out through the mouth and nose. Despite partial aspiration of the fluid, and the application of supportive measures, he lived only 5 more hours. (Chart 1.)

*Results of Clinical Laboratory Procedures**(a) Leucocyte Counts.—*

Date.....	Oct. 28	Oct. 31	Nov. 2	Nov. 5	Nov. 6
No. per c.mm. of blood.....	10,600	7,150	9,900	17,900	14,750
Polymorphonuclears, <i>per cent.</i>	67	60	50	71	84
Metamyelocytes I, <i>per cent.</i>			1	5	
Metamyelocytes II, <i>per cent.</i>			15	6	
Monocytes, <i>per cent.</i>	7	12	6	5	
Lymphocytes, <i>per cent.</i>	26	26	25	13	16
Eosinophiles, <i>per cent.</i>		2	3	0	

(b) *Blood Cultures.*—Blood cultures were taken on Nov. 4, 5, and 8; aerobic and anaerobic cultures were made and media suitable for growth of spirochetes were used. All were negative.

(c) *Lesions on Fingers.*—Direct smear revealed only a few cocci and many pus cells; dark-field examination was negative. Swab cultures yielded *Staphylococcus albus* (predominating) and *Streptococcus hemolyticus*.

(d) *Spinal Fluid.*—Cultures sterile; other findings already recorded.

(e) *Animal Inoculations.*—Two mice, two rats, two guinea pigs, and two rabbits were injected intraperitoneally with blood. One *Macacus rhesus* monkey was inoculated with the exudate from the lesion on the fingers (about 0.05 cc.) into the testicle, with 1 cc. of the spinal fluid intracerebrally and 0.5 cc. subcutaneously, and with 10 cc. of blood intraperitoneally. No significant results were obtained.

Abstract of Pathological Findings

Necropsy was performed by Thomas A. Gonzales, Deputy Chief Medical Examiner of New York City. Very few gross pathologic changes were found. In the left axilla there were several solitary enlarged lymph nodes the substance of which was moderately hemorrhagic. The spleen was rather soft in consistency; the pulp was deep red and the follicles were somewhat prominent. The urinary bladder extended midway to the umbilicus. The cortical convolutions of the brain appeared flattened. On section, the brain and the cord in the cervical, thoracic, and lumbar regions showed no gross lesions except that the cord was somewhat edematous.

The histological picture of the midcervical and upper dorsal regions of the spinal cord was that of an acute transverse myelitis. An inflammatory exudate of mononuclear cells, mostly perivascular, was present in the white matter as well as in the gray matter of the cord. The severest reaction in the gray matter appeared to be in the middle part and the base of the posterior horns. In the white matter of the cord there was a good microglial reaction but only a few of these cells had reached the stage of compound granular corpuscles. Sections stained for

neuroglia showed no proliferation of the astrocytes. Sections stained for myelin showed no tract degeneration in the spinal cord, nor any areas of perivascular demyelination in the central nervous system. The medulla showed a marked inflammatory exudate, particularly severe in the floor of the fourth ventricle; this exudate was not mononuclear but rather predominantly polymorphonuclear with many large groups of cells suggesting abscess formation. The pons, the basal ganglia, internal capsule, and uncinate gyrus showed marked perivascular infiltration, mostly with round cells. The frontal lobe showed some small but quite definite hemorrhagic foci just beneath the surface. There was a mononuclear exudate about many of the vessels of the pia. The left brachial plexus showed no evidence of an inflammatory exudate nor of any myelin degeneration. (Figs. 1 and 2.)

Significant microscopic changes were observed also in the regional lymph nodes, the spleen, and the adrenals. Examination of the regional lymph nodes (left axilla) showed intense hyperemia and focal areas of hemorrhage. Beneath the capsule and for a short distance within the substance of the node, small foci of necrosis were seen. (Fig. 3.) Numerous sections stained by Twort, Gram-Weigert, and Ziehl-Nielsen methods, revealed no organisms. The spleen showed hyperemia and a few areas of necrosis similar to those seen in the lymph nodes. Sections through both adrenals revealed the presence of several large confluent necrotic areas, similar to those found in the lymph nodes and spleen. Those in the adrenals, however, were surrounded by a moderate number of leucocytes; bacterial stains revealed no organisms.¹ (Fig. 4.)

Clinical and Pathological Manifestations

Clinically there presented itself at the site of the monkey bites a mild and relatively insignificant cellulitis of the fingers which was followed by a mild regional lymphangitis and lymphadenitis. 13 days after the bite and 10 days after the first signs of local inflammation, there appeared the typical picture of a transverse myelitis which ascended and resulted in death from respiratory paralysis within 4 days. Clinically there were practically no manifestations pointing to an involvement of the brain. The pathological changes in the central nervous system were diffuse but most marked in the medulla and spinal cord. The predominance of mononuclear cells in the exudate, the perivascular round cell infiltration, and the other changes

¹ The authors are indebted to Dr. Thomas A. Gonzales for the report on the gross pathology, to Dr. Lewis Stevenson for the report on the microscopic pathology of the nervous system, and to Dr. Irving Graef for that of the microscopic pathology of the other organs.

which were described, are pathological findings which are considered characteristic of virus diseases of the central nervous system in general, yet not characteristic of any virus disease in particular. It is only from the distribution of the pathologic process that one can perhaps exclude acute anterior poliomyelitis on a histopathologic basis alone; the absence of perivascular demyelination which characterizes the so called acute disseminated encephalomyelitis, or postinfectious encephalomyelitis would serve to exclude this condition as well. The absence of any pathologic reaction in the regional brachial plexus, which would indicate the passage or the presence of the infectious agent, may have some bearing on the determination of the portal of entry of the infectious agent into the central nervous system and the pathogenesis of the disease. It becomes extremely important therefore to take note of the focal areas of necrosis which were observed in the regional lymph nodes, the spleen, and the adrenals. For the possibility, and forthcoming evidence, that these were caused by the same virus as that which injured the central nervous system, may not only elucidate the pathogenesis of this disease, but also serve to amalgamate these various organic changes into one pathological, and perhaps clinical, entity.

ISOLATION OF THE INFECTIOUS AGENT AND REPRODUCTION OF THE DISEASE IN ANIMALS

The material available for study consisted of pieces of brain, medulla, spinal cord, spleen, and regional lymph node which were obtained at necropsy 5 hours after death and preserved in 50 per cent glycerine. Although the microscopic pathology of the organs was unknown at the time this study was begun, the sterility of the blood and spinal fluid cultures taken during life, suggested the possibility that the causative agent belonged to the group of filtrable viruses. Since only a limited number of animals was available, portions of the brain, medulla, and spinal cord were pooled into one mixture and for most experiments the spleen and regional lymph node into another. 10 per cent emulsions of these pooled mixtures were prepared in the usual manner. Other emulsions prepared for different tests from tissue which had been in glycerine for periods varying from a few days to several weeks, proved unsterile on culture; direct

smear of the emulsions revealed no organisms but cultures on agar and broth, or incubation of the saline emulsion itself, invariably resulted in an almost pure growth of Gram-negative bacilli. Studies of these bacilli indicated that they belonged to the colon group. *Macacus rhesus* monkeys, dogs, guinea pigs, mice, and rabbits were injected with the emulsions by various routes.

Transmission Experiments on Macacus rhesus Monkeys

Brain and Cord.—Monkey A received 2 cc. intracerebrally and 20 cc. intraperitoneally of a freshly prepared 10 per cent emulsion of the brain and cord. Daily observations on the condition and temperature of the monkey revealed no abnormal changes. The monkey died on the 11th day after injection. Post-mortem examination of the brain and cord as well as of the other organs revealed no significant findings; the histologic sections were unsatisfactory. Cultures taken from the brain, cord, heart's blood, and peritoneum were sterile. The brain and cord of this monkey were preserved in glycerine, and 8 days later a saline emulsion was prepared from them and another *Macacus rhesus* monkey B was inoculated intracerebrally and intraperitoneally; this monkey had fever on the 4th and 5th days after injection, transitory slight tremors, but remained in good condition over a period of 2 months' observation. In view of the fact that the results from Monkey A were inconclusive, another *Macacus rhesus* monkey C was inoculated intracerebrally and intraperitoneally with a freshly prepared emulsion of glycerinated tissue of the original brain and cord. When Monkey C showed neither fever nor any other abnormal signs for 3 weeks, it was reinoculated intracerebrally and intraperitoneally with another freshly prepared emulsion of the original brain and cord. This monkey remained well for 2 months after the last injection.

Spleen and Regional Lymph Node.—Monkey D was inoculated intracerebrally (2 cc.) and intraperitoneally (20 cc.) with a freshly prepared 10 per cent emulsion of the glycerinated spleen and regional lymph node; when after 2 weeks it failed to show either fever or abnormal signs, it was reinoculated in the same manner with another freshly prepared emulsion. Monkey D remained well.

From these tests it appears extremely unlikely that either the brain and cord or the spleen and regional lymph node contained any agent capable of inciting a specific disease in the *Macacus rhesus* monkey.

Experiments on Mice, Guinea Pigs, and Dogs

Mice, guinea pigs, and dogs were injected intraperitoneally and intracerebrally with emulsions of the original brain and cord, as well as of the spleen and lymph node. No evidence of a virus disease was elicited in any of these animals, but

they succumbed to an infection with a Gram-negative bacillus present in the patient's organs, and the disease thus produced in no way resembled that from which the patient died. The organism was a member of the colon group, as already mentioned, and tests with it in pure culture showed it to be extremely virulent for mice, guinea pigs, and dogs. The bacteriological studies during life as well as the histopathological changes which were present in the patient's organs, leave little doubt that this organism was a postmortem contaminant.

Experiments on Rabbits

The transmission experiments on rabbits were carried out in much the same manner as for the other animals with the exception that the spleen and regional lymph node material were used separately.

Brain and Cord.—As indicated in Table I, of two rabbits (Nos. R 3-51 and R 3-52) which were injected intracerebrally with 0.5 cc. of the emulsion, one survived and the other died on the 3rd day. There were no suggestive signs before death; the postmortem cultures from the brain and heart's blood were sterile; the brain and cervical cord were somewhat congested. 0.5 cc. of a 10 per cent emulsion of the glycerinated brain and cord of the dead rabbit (No. R 3-53) was injected intracerebrally into another rabbit (No. R 3-64). Rabbit R 3-64 had excessive salivation on the 5th, 6th, and 7th days after injection but showed no other signs suggestive of encephalitis; 1 month after injection, this rabbit was retested with active virus. The results will be given elsewhere under the discussion of abortive infections and active immunity.

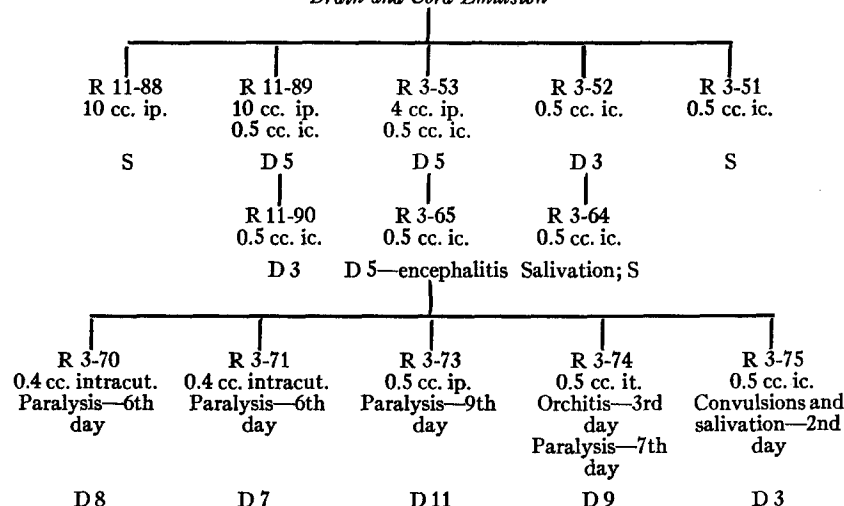
The one rabbit (No. R 11-88), injected intraperitoneally only, with 10 cc. of the original brain and cord emulsion, survived without signs. However, both rabbits (Nos. R 11-89 and R 3-53) which were injected intracerebrally as well as intraperitoneally died on the 5th day, again with sterile heart's blood and brain cultures, and also without any suggestive antemortem signs. As a result of later observations it is not improbable that these rabbits may have had antemortem convulsions but since the interval between the onset of these signs and death may be very short, these might not have been noticed. The brain and cord of each dead rabbit were glycerinated separately, and new rabbits were injected intracerebrally with fresh emulsions prepared from them. As shown in Table I both died—one on the 3rd day and one (No. R 3-65) on the 5th day. Rabbit R 3-65 was seen before death, and convulsions as well as excessive salivation were observed. The postmortem cultures of the heart's blood and brains of these rabbits were again sterile.

It seemed evident that the brains and cords of the rabbits that died as result of inoculation contained some virus which was capable of inducing an encephalitis and of being transmitted in series. The

question arose now whether or not it would be possible to reproduce the disease under investigation, by introducing the virus in a focus outside the central nervous system.

The glycerinated brain and cord of No. R 3-65 were used in these tests. Two rabbits (Nos. R 3-70 and R 3-71) were injected intracutaneously with a fresh 10 per cent emulsion, one rabbit (No. R 3-73) intraperitoneally, one (No. R 3-74)

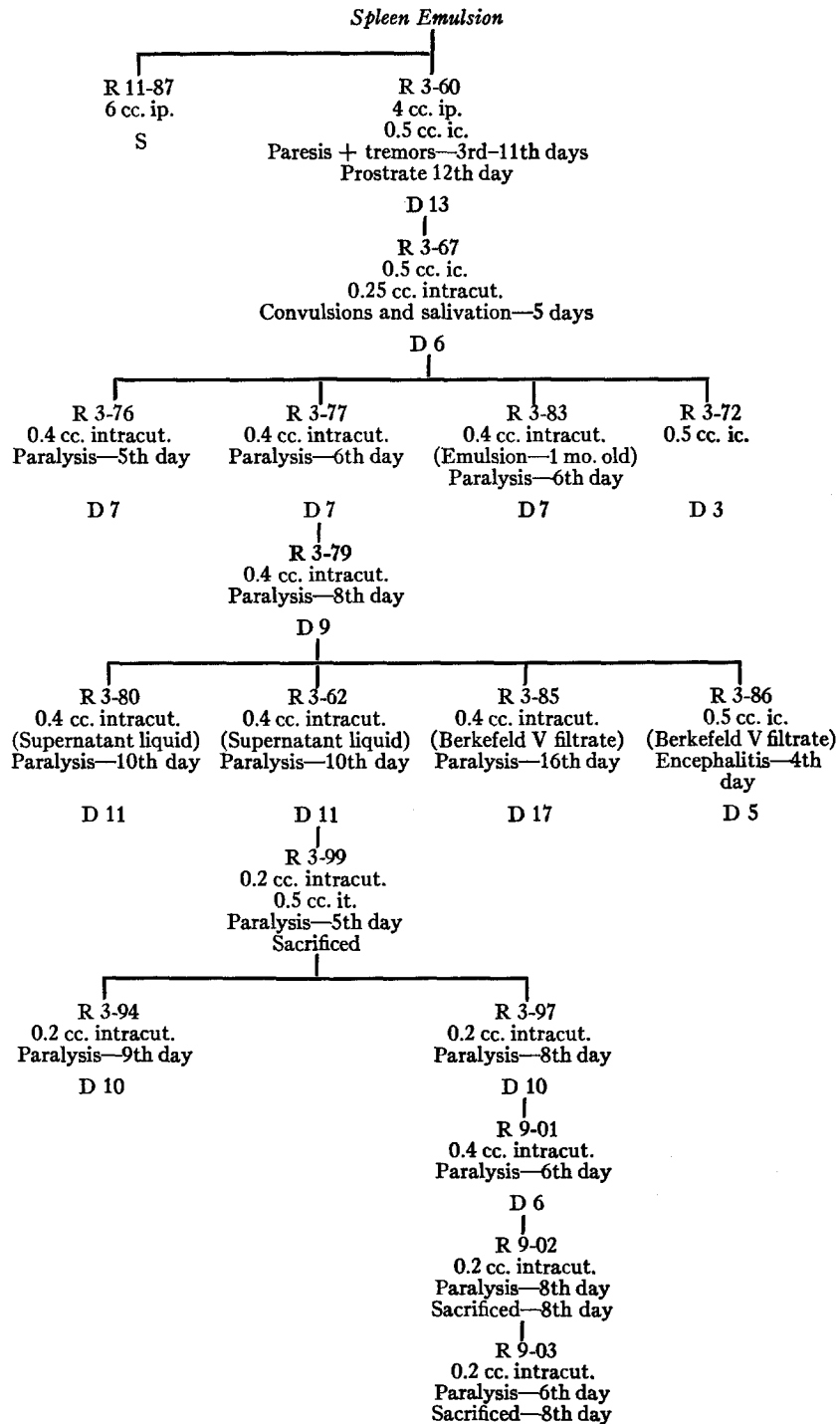
TABLE I
*Transmission Experiments in Rabbits with Human Brain and Cord
Brain and Cord Emulsion*



In Tables I and II, ip. indicates intraperitoneal; ic., intracerebral; it., intratesticular; intracut., intracutaneous; D 5, dead on 5th day after injection; S, survived.

intratesticularly, and still another (No. R 3-75) intracerebrally. The rabbit injected intracerebrally developed convulsions 2 days later and died on the 3rd day. All the other rabbits, however, developed paralysis of the posterior extremities after a definite incubation period as indicated in Table I; this paralysis progressed more or less rapidly to involve the anterior extremities, the rabbits dying of respiratory failure without convulsions and with salivation occurring only immediately before death or not at all. A more detailed description of the course of events will be given in another part of this paper. The resemblance of the course of this disease in rabbits to that which was observed in the patient was most remarkable.

TABLE II
Transmission Experiments in Rabbits with Human Spleen



Spleen.—Table II reveals that the transmission experiments with the original spleen emulsion yielded a virus which behaved in precisely the same manner as that which was shown to be present in the original brain and cord. It will again be noted that in the first passage intraperitoneal injection alone failed to give results whereas the combined intracerebral and intraperitoneal injection resulted in a take which permitted of ready subsequent transmission.

The first passage rabbit (No. R 3-60) manifested no characteristic signs and lived for 13 days; the second passage rabbit (No. R 3-67), however, injected with the brain and cord of No. R 3-60, developed typical signs of encephalitis on the 5th day and died on the 6th. The postmortem smears and cultures of the heart's blood and brains of these rabbits were sterile. The intracutaneous injection of a 10 per cent emulsion of this brain and cord (No. R 3-67) resulted in the typical development of first, paralysis of the posterior extremities with rapid progression cephalad and death by respiratory paralysis, without convulsions and little or no salivation. The brains and cords of these rabbits were again capable of reproducing this disease in precisely the same manner when injected intracutaneously.

By these experiments a virus, transmissible in series, was shown to be present in the human spleen, as well as in the brain and cord, and capable of inducing a disease in rabbits, clinically similar to the human disease. This virus has undergone fifteen serial passages now, and from the second passage onward has behaved like a fixed virus.

Regional Lymph Node.—The attempt to isolate a virus from the small piece of the regional lymph node which was available proved unsuccessful. One rabbit injected intracerebrally with the emulsion died on the 6th day without any signs other than diarrhea. The postmortem cultures were sterile, but another rabbit injected with an emulsion of its brain and cord survived 1 month without any signs.

Nature of the Virus

For the purpose of reference in subsequent discussions, the virus isolated from spleen, brain, and cord of the human case, will be called the B virus.

(a) Disease Produced in Rabbits Following the Introduction of the B Virus by Various Routes

Intracerebral Route.—The course of the experimental rabbit disease following the introduction of the virus directly into the brain differed considerably from that

which followed its inoculation into foci outside the central nervous system. During the first 48 hours after the intracerebral injection of 0.5 cc. of a 10 per cent fresh emulsion of glycerinated brain and cord, the rabbit appears entirely normal and the temperature shows no abnormal variation; within the next 12 to 24 hours the temperature may or may not rise and in rapid succession there appear generalized convulsions, increased salivation, and death.

Intracutaneous Route.—The routine procedure consisted of injecting 0.2 cc. of the 10 per cent brain and cord emulsion into two places (0.4 cc. altogether) on one

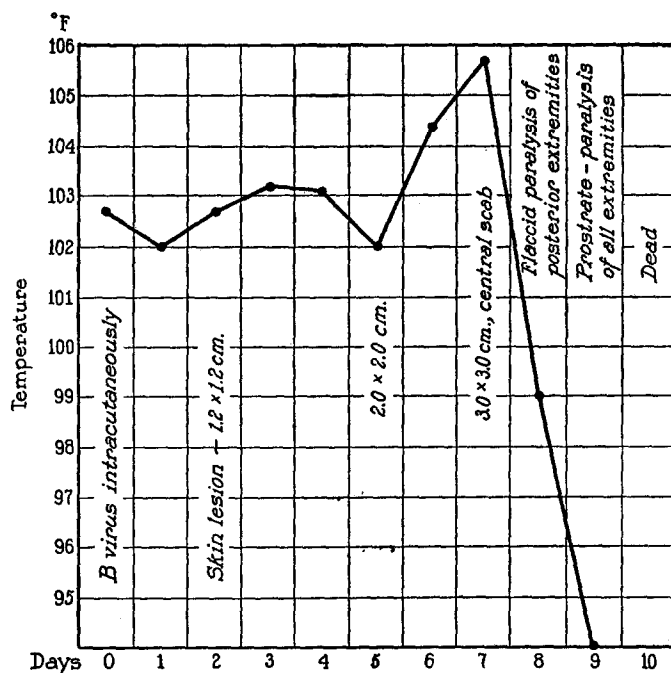


CHART 2. Temperature of rabbit injected intracutaneously with B virus.

side of the abdomen or back from which the hair was clipped. There was almost no skin reaction in the first 24 hours. Within the next 24 hours erythematous papules varying from 1 to 2 cm. in diameter appeared at the site of inoculation. The following day there was usually some hemorrhagic necrosis in the center of the papule which became more marked for another day and then proceeded to heal. About the 6th day after injection there was usually a rise in temperature (not always observed, however) and paralysis of one or both of the posterior extremities appeared. The side which was paralyzed first bore no relationship to the side receiving the intracutaneous injections. Within 12 to 24 hours the paralysis usually progressed cephalad to involve the fore limbs. The rabbit either died

within 24 hours of the onset of paralysis or lingered on in a prostrate condition with slow, gasping respirations (sometimes only once or twice a minute) for another 24 hours; but thus far, not a single rabbit which developed paralysis has survived. With one exception of uncertain nature, the paralysis which followed the intracutaneous injection of the virus was always flaccid and was not associated with convulsions. In some of the rabbits there was twitching of the facial muscles or convulsive movements of the head and slight salivation just before death; and in some a relaxation of the sphincters with almost continuous dribbling of urine was associated with the paralysis. In view of the fact that postmortem examinations revealed a markedly distended urinary bladder in most rabbits, it is highly probable that the dribbling of urine may be due to a relaxation of the sphincter secondary to urinary retention. (Chart 2.)

The resemblance of the disease produced in rabbits to that observed in the human case is most striking. A local, relatively insignificant, cutaneous lesion is followed after an interval by flaccid paralysis of the hind limbs associated apparently with urinary retention, and there is a cephalad progression of the process and death by respiratory failure.

Intratesticular Route.—The course of the disease which followed the intratesticular injection of the B virus can be best illustrated by a protocol.

Protocol of Rabbit R 3-74.—Inoculation: 0.5 cc. of 10 per cent emulsion of rabbit brain and cord (2nd generation passage virus) into left testicle.

Jan. 21, 1933. 103.5°F. Just before injection.

Jan. 22. 102.2°F. No apparent reaction.

Jan. 23. 103.0°F. No apparent reaction.

Jan. 24. 106.3°F. Marked swelling of left testicle.

Jan. 25. 103.2°F. Brawny induration of left testicle.

Jan. 26. 105.2°F. Testicle very hard and brawny; about three times natural size.

Jan. 27. 105.0°F. Same.

Jan. 28. 104.1°F. 2 p.m. Diminution in size of swollen testicle. Tremors and partial paralysis of right posterior extremity. 11 p.m. Complete flaccid paralysis of both posterior extremities; partial paralysis of right anterior extremity; cannot get up; no salivation.

Jan. 29. 95.6°F. 1:30 p.m. Condition same; paralysis definitely flaccid. Loss of sphincter control—feces and urine dripping continually. No salivation; slight retraction of head.

Jan. 30. Temperature unobtainable. Prostrate; gasping for air—only occasional breath. Lived on thus all day; slight salivation towards end. Died at night.

It will be seen from the protocol that the intratesticular injection of the virus resulted in an orchitis associated with fever on the 3rd day, and the development of typical flaccid paralysis on the 7th day. Although the virus was injected into the left testicle, it was the right hind limb which was paralyzed first. An ascending myelitis, rather than an encephalitis, invariably followed the intratesticular injection of the B virus.

Intraperitoneal Route.—For 8 days following the intraperitoneal injection of 0.5 cc. of the same virus emulsion into a rabbit no abnormal changes either in the temperature or physical status could be observed. On the 9th day there was a rise in temperature and in the evening of that day partial paralysis of both posterior extremities was present. On the 10th day the temperature had dropped to a subnormal level, and there was complete paralysis of the posterior extremities and loss of sphincter control (postmortem examination revealed a markedly distended urinary bladder); there was no salivation. Death occurred on the 11th day with evidence of antemortem salivation.

Corneal Route.—All attempts to implant the virus on the cornea of rabbits proved unsuccessful. Emulsions of the original brain and cord (human) were used as well as passage virus. There was neither local keratitis nor any demonstrable systemic invasion. It will perhaps be interesting to note here that whereas intracutaneous injection of the virus always caused the disease, there was no apparent reaction to virus introduced on the scarified skin. All the rabbits which were used for the corneal implantation tests were subsequently proved to be susceptible to the virus.

(b) *Pathology of the Experimental Disease*

The gross pathology of all the dead rabbits was recorded as routine and in a few selected instances the microscopic pathology was studied. Necropsy on twelve rabbits which died following intracerebral inoculation of the virus showed no obvious gross pathological change other than slight to moderate congestion of the brain and the cervical portion of the spinal cord. In contrast to the findings in the rabbits which died following intracutaneous injection of the virus, no lesions were discernible in any of the abdominal viscera. The rabbits which were injected intracutaneously, intraperitoneally, and intratesticularly showed, in addition to the congestion of the spinal cord and brain, the following grossly apparent changes in the abdominal viscera. The spleen was enlarged to twice or three times its usual size in almost every case; in many instances, grayish white spots, about 1 to 2 mm. in diameter, were seen just beneath the capsule and on section in the central portion of the parenchyma. In a few rabbits there was a congestion and mottling of the adrenals visible in the gross, and lesions were found with the microscope in other adrenals that appeared normal. The liver,

in many instances, showed the same grayish white spots that were observed in the spleen. In approximately 50 per cent of the rabbits the urinary bladder was found to be markedly distended. No pathologic changes were observed in the heart, lungs, and kidneys.

The evaluation of the microscopic changes produced by the virus in the central nervous system of rabbits, proved to be difficult on account of the frequency with which lesions characteristic of *Encephalitozoon cuniculi* were encountered. The brains and cords of three rabbits which died following intracutaneous injection of the virus showed no *Encephalitozoon cuniculi* lesions; in these there was no perivascular cellular infiltration but instead a pericellular and perivascular edema of the type which Brown and Symmers (2) described in certain human cases which they called acute serous encephalitis. There was no meningeal reaction. The most prominent change was present in the spinal cord and consisted of neuron damage as evidenced by varying degrees of necrosis, hyperchromia, vacuolization, nuclear degeneration, and by a slight, rather diffuse infiltration with mononuclear cells. Definite characteristic inclusion bodies of the type observed in the other organs were not encountered, but various types of intranuclear bodies were seen in the large cells of the anterior and lateral horns. Stains for myelin revealed no foci of demyelination. The paucity of microscopic lesions may perhaps be explained by the fact that only 24 to 48 hours intervened between the onset of neurologic signs and death. (Figs. 5 to 7.)

Striking microscopic changes were observed in the skin, adrenals, spleen, liver, and injected testicle. Sections through the skin lesion (at the site of inoculation), stained with hematoxylin and eosin, showed extensive necrosis of the cutis and subcutaneous tissue with a moderate infiltration of polymorphonuclear and mononuclear cells. One Giemsa-stained section of a skin lesion (tissue obtained after death) showed typical eosinophilic intranuclear inclusion bodies in the epithelial cells at the periphery of the lesion (Fig. 13). One of the skin lesions was excised 48 hours after injection of the virus; careful search of the Giemsa-stained section failed to reveal any inclusion bodies although definite necrosis was present. Characteristic areas of necrosis of varying size were found in all the adrenals examined except those obtained from a rabbit which was injected with the virus intracerebrally. The areas of necrosis were found chiefly in the cortex and were not surrounded by a zone of cellular infiltration (Fig. 8). Examination of Giemsa-stained sections revealed many typical eosinophilic intranuclear inclusion bodies in the cortical cells surrounding the necrotic zones (Fig. 9). The spleen showed focal necrosis with a few intranuclear inclusion bodies in endothelial cells (Fig. 12). The liver similarly showed numerous foci of necrosis (Fig. 10); eosinophilic intranuclear inclusion bodies were found in liver cells and although more numerous than in the spleen, they were not as abundant as in the adrenals (Fig. 11). The injected testicle showed widespread necrosis and cellular infiltration but insufficient work was done on the demonstration of inclusion bodies. The intranuclear inclusion bodies which were observed in the organs mentioned differed in no way from those described for herpes, varicella, Virus III disease, or salivary gland disease of guinea pigs.

The chief pathologic manifestations of the experimental virus disease in rabbits present many features in common with those in the human disease. The occurrence of focal necrosis in the adrenals and spleen in addition to central nervous system involvement is a striking feature in both. But although the visceral lesions resemble each other strongly, the pathologic picture in the central nervous system is not precisely the same. However, the difference of species or the shorter duration of the experimental disease may perhaps be responsible. In experimental poliomyelitis in the monkey, the microscopic pathology may vary with the virulence of the virus—the more virulent virus producing a less intense inflammatory reaction and a more rapid death.

(c) Filtrability of the B Virus

Although the failure to demonstrate any microscopically visible organisms either directly or on ordinary culture media in the tissues and fluids of rabbits dying from the experimental disease, and the successful transmission of the disease in series, together with the production of typical intranuclear inclusion bodies, left little doubt that the causative agent can be grouped with the so called filtrable viruses, it was nevertheless important to determine its filtrability.

Some of the "filtrable viruses" pass through the ordinary bacteriological filters with great difficulty or not at all. Ward and Tang (3) demonstrated that by emulsifying herpes virus in broth instead of saline, the virus could be recovered consistently from a Berkefeld V filtrate. The emulsions for the present tests were prepared in broth of pH 7.6. Two sets of tests were performed: one with a Seitz filter and the other with a new Berkefeld V candle, using negative pressure in each case. The period of filtration with the Seitz filter was 3 minutes, and with the Berkefeld V candle, 40 seconds; the broth emulsion was centrifuged at high speed and the supernatant liquid was used for filtration as well as for the control inoculations.

The results of the tests are shown in Table III. The rabbit injected intracerebrally with the Seitz filtrate failed to develop any signs. The filtrate from the Berkefeld V candle, however, produced the typical experimental disease, both on intracutaneous and intracerebral inoculation. The increased incubation period, however, suggested that there was less virus in the filtrate than in the highly centrifuged

supernatant liquid from which it was derived. The Berkefeld V filtrate showed no growth on ordinary culture media; the same candle

TABLE III
Filtrability of B Virus

Material tested	Type of filter	Rabbit No.	Route of inoculation	Dose cc.	Results
3rd generation passage virus. 10 per cent emulsion of brain and cord in broth	Unfiltered supernatant liquid	R3-79	Intracutaneous	0.4	Skin lesion—48 hrs.; paralysis—8th day; dead—9th day
	Seitz	R3-78	Intracutaneous	0.4	No skin lesion or paralysis; died—12th day of intercurrent infection
	Seitz	R3-80	Intracerebral	0.5	Excessive salivation and spasticity—12th, 13th, and 14th days; recovered; reinoculated 33 days after injection
4th generation passage virus. 15 per cent emulsion of brain and cord (No. R3-79) in broth	Unfiltered supernatant liquid	R3-80	Intracutaneous	0.4	Practically no skin lesion; paralysis—10th day; dead—11th day
	Unfiltered supernatant liquid	R3-62	Intracutaneous	0.4	Skin lesion—6th day; fever and increase in skin lesion—7th, 8th, and 9th days; paralysis—10th day; dead—11th day
	New Berkefeld V	R3-85	Intracutaneous	0.4	No skin lesion; paralysis—15th day; dead—16th day
	New Berkefeld V	R3-86	Intracerebral	0.5	Fever, paresis and complete paralysis of left anterior extremity—4th day; salivation and death—5th day

was used subsequently for the filtration of pneumococcus broth cultures and yielded sterile filtrates.

(d) *"Viability" of Saline Suspensions of the Virus*

Since most viruses deteriorate rather rapidly when not preserved in glycerine or by other dehydrating procedures, it is important to record the fact that a 10 per cent saline emulsion of a third generation passage virus (rabbit brain and cord) produced the typical disease upon intracutaneous injection just as well and with the same incubation period after the emulsion had been in the ice chest at 5°C. for 1 month, as it did when freshly prepared. This test was originally performed with the idea that the virus might be sufficiently attenuated by this procedure to permit its use in immunity experiments.

(e) *Abortive Infections and Active Immunity*

It is characteristic of most virus diseases that one attack, abortive or otherwise, imparts immunity to the surviving animal. The study of the immune phenomena with the B virus was important not only for itself but as an aid to establishing its identity and relationship to other known viruses.

Two rabbits had what may perhaps be called abortive attacks. One (No. R 3-80) was injected intracerebrally with a Seitz filtrate of an active virus emulsion and developed only spasticity and excessive salivation on the 12th, 13th, and 14th days but recovered completely; but upon reinoculation intracutaneously with active virus 33 days after the first injection, it developed typical paralysis and death occurred even though no skin lesion resulted at the site of inoculation. The other (No. R 3-64) was injected intracerebrally with a brain and cord emulsion of first passage virus (No. R 3-52) which produced only excessive salivation on the 5th, 6th, and 7th days; but 1 month after the first injection it was reinoculated intracerebrally with active virus, developed typical signs of encephalitis, and died. Another rabbit which after scarification of the cornea had virus introduced into the conjunctival sac on several occasions and failed to develop keratitis, succumbed in a typical manner after intracutaneous injection of active virus. Rabbit R 3-51 which failed to develop any signs after the intracerebral injection of the original (human) brain and cord, later succumbed typically to the intracutaneous injection of active virus.

In appraising these results it must be remembered that not a single rabbit which developed the typical experimental disease survived. More work will be necessary with graded doses of virus in the attempt to produce non-fatal attacks as well as in the testing for acquired

resistance, before any definite statement can be made about the development of immunity.

Relation of the B Virus to the Known Viruses

During the course of the present study the question naturally arose as to whether the B virus was a strain of an already known virus or whether it had not hitherto been described. The fact that the original material failed to take in *Macacus rhesus* monkeys and dogs whereas a characteristic disease was reproduced in rabbits, would seem to exclude the viruses of poliomyelitis and rabies as we know them. They can also be excluded by the type of inclusion body which the B virus induced and the absence of Negri bodies in its case. The necrotic lesions which were found in the adrenals in the present study are very like the lesions in the adrenals produced experimentally by the viruses of vaccinia (4) and herpes (5). The typical intranuclear inclusion body produced by the B virus as well as the absence of the Guarnieri bodies of vaccinia, would appear to limit the known possibilities to those viruses which give rise to the same type of inclusion body; *i.e.*, herpes, varicella, Virus III disease of rabbits, salivary gland disease of guinea pigs, and so called visceral disease (6). Of this group of viruses only two need be considered; namely, Virus III disease (a spontaneous disease of rabbits) and herpes. Although Virus III must be considered and guarded against whenever the experimental reproduction of any disease is attempted in rabbits, the circumstances under which the B virus was isolated, the regularity with which the human organs repeatedly infected rabbits from different sources, the striking similarity of the experimental and human disease, as well as the dissimilarity with that which Virus III is known to induce, all point against its identity with the B virus. The exclusion of Virus III disease leaves only the virus of herpes for consideration. Certain strains of herpes encephalitis virus resemble the B virus in the following respects: (a) localization in the central nervous system following intracutaneous injection, (b) morphologically similar necrotic lesions in the adrenals following intracutaneous injection, and (c) similar intranuclear inclusion bodies. There are almost as many differences between the two, however: (a) although the herpes virus may produce myelitis when injected into zones supplied by nerves which enter the

spinal cord (7), it produces an encephalitis primarily when injected intratesticularly (7-9), whereas the B virus invariably attacks the spinal cord first, as evidenced by the flaccid paralysis of the posterior extremities and urinary retention, with cephalad progression of the lesion and death by respiratory failure, (b) to cause focal necrosis of the spleen and liver a direct injection of herpes virus must be made into these organs (7), whereas these lesions are a part of the systemic disease following the intracutaneous injection of the B virus, (c) in the present study it has been impossible to produce keratitis with the B virus. The final determination of the identity or distinctness of the two viruses must depend upon cross-immunity tests. Unfortunately, no rabbits have recovered from infection with the B virus thus far nor has an immune rabbit been secured by any method attempted. Attempts to immunize rabbits to intracerebral inoculations with virulent herpes virus in order to test them for immunity to the B virus have been unsuccessful. However, it would appear that the B virus possesses certain characteristic properties which justify its consideration as a distinct entity.²

SUMMARY

A case of acute ascending myelitis which followed the bite of an apparently normal *Macacus rhesus* monkey is described. The clinical course as well as the pathological changes has been studied and found to be suggestive of a virus cause for the disease. The absence of perivascular demyelination removes the case from the realm of acute disseminated encephalomyelitis and establishes it more or less definitely as a primary acute infectious myelitis. An extremely important feature of the pathological picture of this disease has been the presence of focal necrosis in the viscera (spleen, adrenals, regional lymph nodes).

Attempts to transmit the disease to *Macacus rhesus* monkeys, dogs, mice, and guinea pigs, employing glycerinated organs from the human

² Through Dr. Josephine B. Neal, Dr. Gay and Dr. Holden obtained from us some brain and cord from the human case. In a preliminary paper (*Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 1051, Case 4) they report the demonstration of a virus having the properties of our B virus as here described and state their belief on experimental evidence that this virus is identical with the herpes virus.

case, proved unsuccessful. By the inoculations of rabbits the presence of a strongly neurotropic, filtrable virus was demonstrated in the patient's brain, cord, and spleen. Following intracutaneous injection of it as derived either from brain and cord or spleen, an experimental disease develops in rabbits which strikingly resembles the human disease in the character of the local lesion, the incubation period, development of urinary retention, and flaccid paralysis of the posterior extremities with cephalad progression, death by respiratory failure, and finally by the occurrence of focal necrosis in the spleen, adrenals, and liver. In attempting to establish the identity of this virus, (the B virus), a consideration of its biological properties excludes the viruses of poliomyelitis, rabies, vaccinia, Virus III disease of rabbits, and the other viruses which are known to produce similar intranuclear inclusion bodies, except perhaps herpes. Although the relationship between the B virus and the virus of herpes must still be determined by cross-immunity tests it has been shown to possess certain properties which warrant consideration of it as a distinct entity.

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REFERENCES

1. Hurst, E. W., and Pawan, J. L., *Lancet*, 1931, **2**, 622.
2. Brown, C. L., and Symmers, D., *Am. J. Dis. Child.*, 1925, **29**, 174.
3. Ward, H. K., and Tang, F., *J. Exp. Med.*, 1929, **49**, 1.
4. Douglas, S. R., Smith, W., and Price, L. R. W., *J. Path. and Bact.*, 1929, **32**, 99.
5. Smith, W., *J. Path. and Bact.*, 1931, **34**, 493.
6. von Glahn, V. C., and Pappenheimer, A. M., *Am. J. Path.*, 1925, **1**, 445.
7. Goodpasture, E. W., and Teague, O., *J. Med. Research*, 1923-24, **44**, 139.
8. Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1925, **41**, 233.
9. Gay, F. P., and Holden, M., *J. Infect. Dis.*, 1929, **45**, 415.

EXPLANATION OF PLATES

PLATE 13

FIG. 1. Cross-section of human spinal cord showing a large area of necrosis; section stained for myelin, Loyez method. $\times 7$.

FIG. 2. Human spinal cord showing extensive cellular infiltration, particularly in white matter; note necrotic state of some of the nerve cells. $\times 135$.

The authors are indebted to Dr. Lewis Stevenson for the photographs shown in Figs. 1 and 2.

FIG. 3. Human axillary lymph nodes showing focal necrosis. Hematoxylin and eosin. $\times 135$.

FIG. 4. Human adrenal showing large necrotic area surrounded by zone of cellular infiltration. Hematoxylin and eosin. $\times 135$.

PLATE 14

FIG. 5. Spinal cord of rabbit injected with B virus intracutaneously; large ganglion cell showing vacuolization, beginning neuronophagocytosis, and nuclear degeneration. Hematoxylin and eosin. $\times 1,160$.

FIG. 6. Same as Fig. 5. Zenker fixation and Giemsa stain. Arrow points to dark red intranuclear body (not a typical inclusion body). $\times 1,160$.

FIG. 7. Same as Fig. 5. Note invasion of gray matter by mononuclear cells and anterior horn cells in various stages of necrosis. Hematoxylin and eosin. $\times 145$.

FIG. 8. Adrenal of rabbit injected with B virus intracutaneously; note large area of necrosis. Hematoxylin and eosin. $\times 145$.

PLATE 15

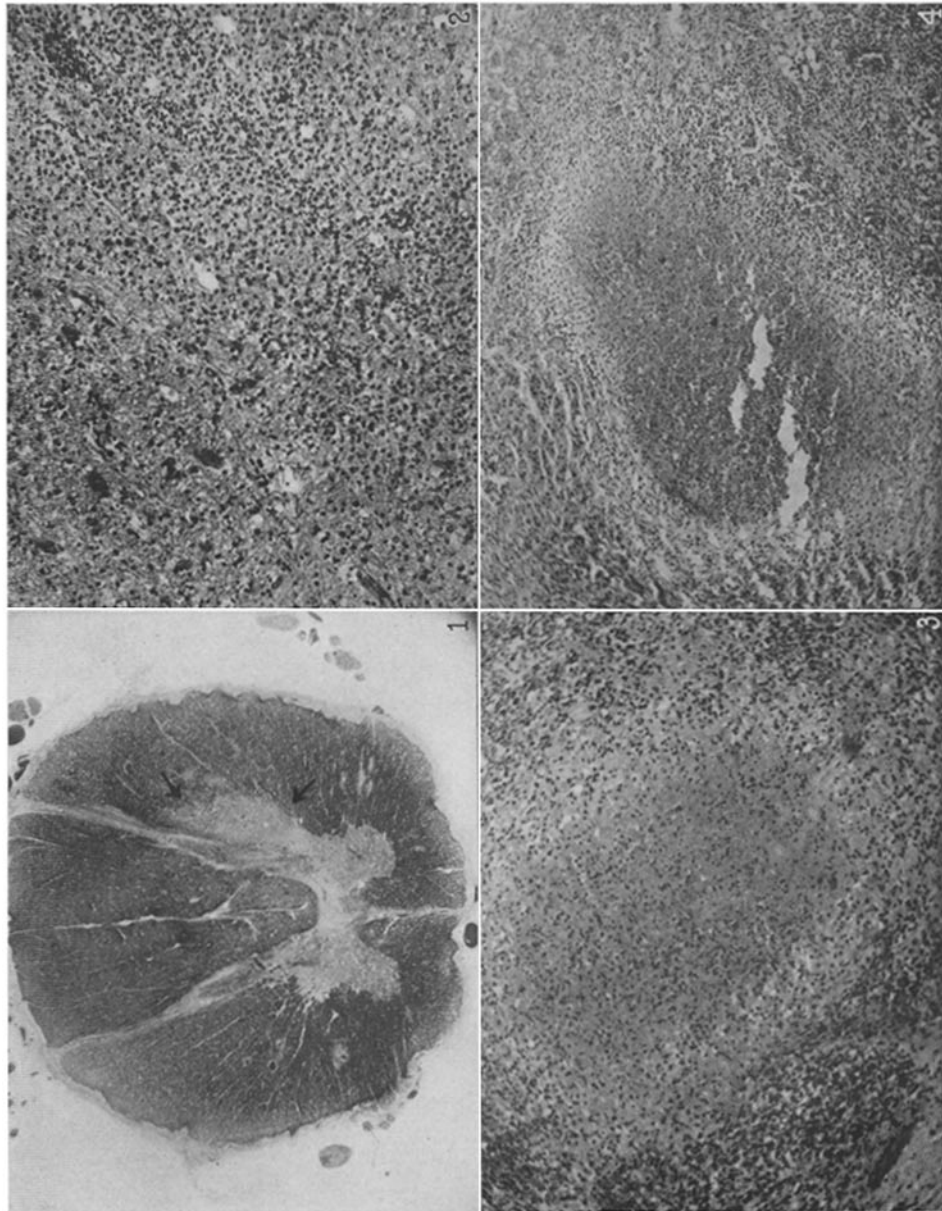
FIG. 9. Same as Fig. 8. Zenker fixation and Giemsa stain. Zone surrounding area of necrosis in adrenal; arrows point to nuclei containing eosinophilic inclusion bodies. $\times 1,210$.

FIG. 10. Liver of rabbit, injected intracutaneously with B virus, showing focal necrosis. Hematoxylin and eosin. $\times 150$.

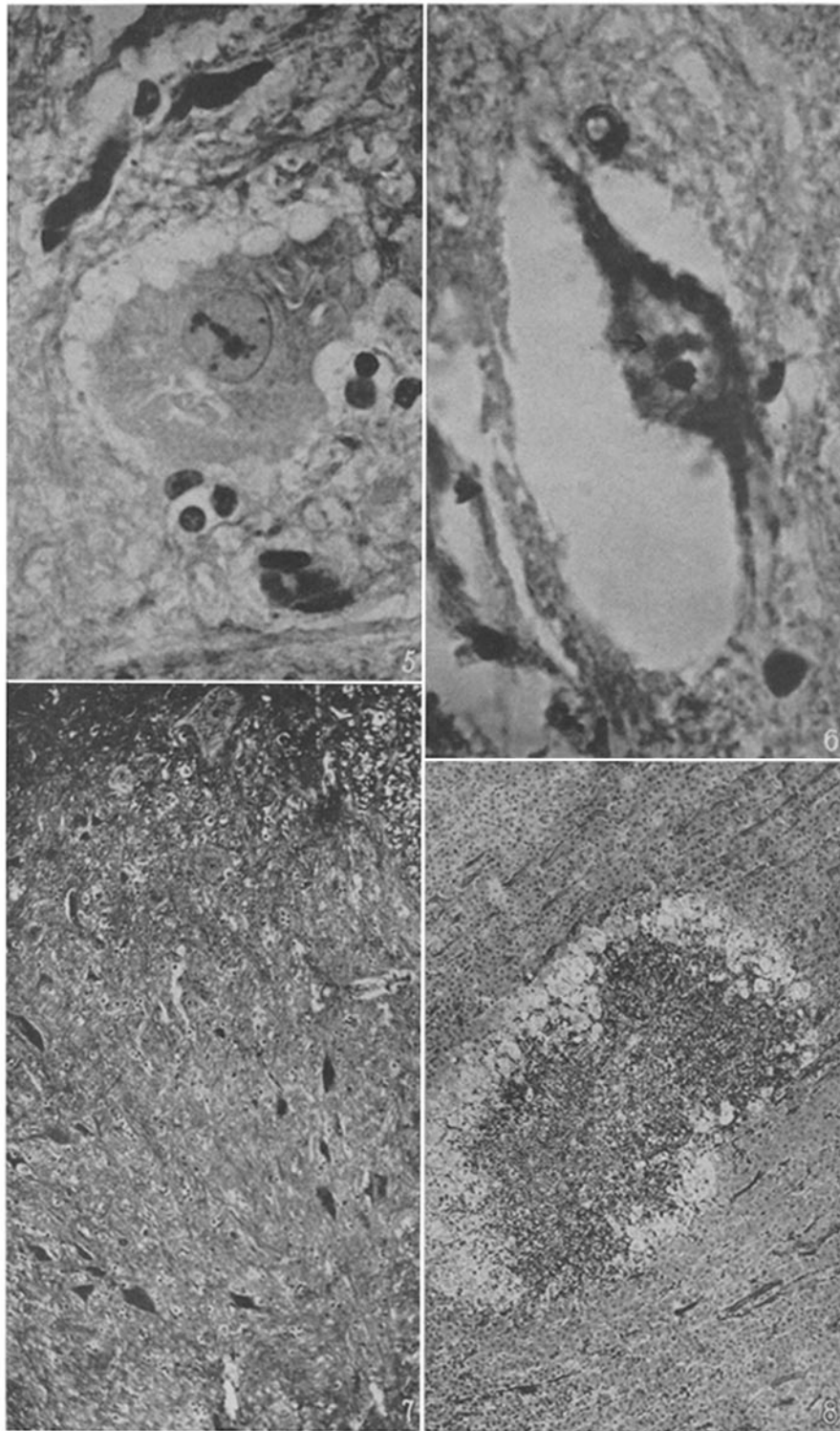
FIG. 11. Same as Fig. 10. Arrow points to nucleus of liver cell containing eosinophilic inclusion body. Hematoxylin and eosin. $\times 1,210$.

FIG. 12. Spleen of rabbit injected with B virus intracutaneously; note focal necrosis. Giemsa. $\times 600$.

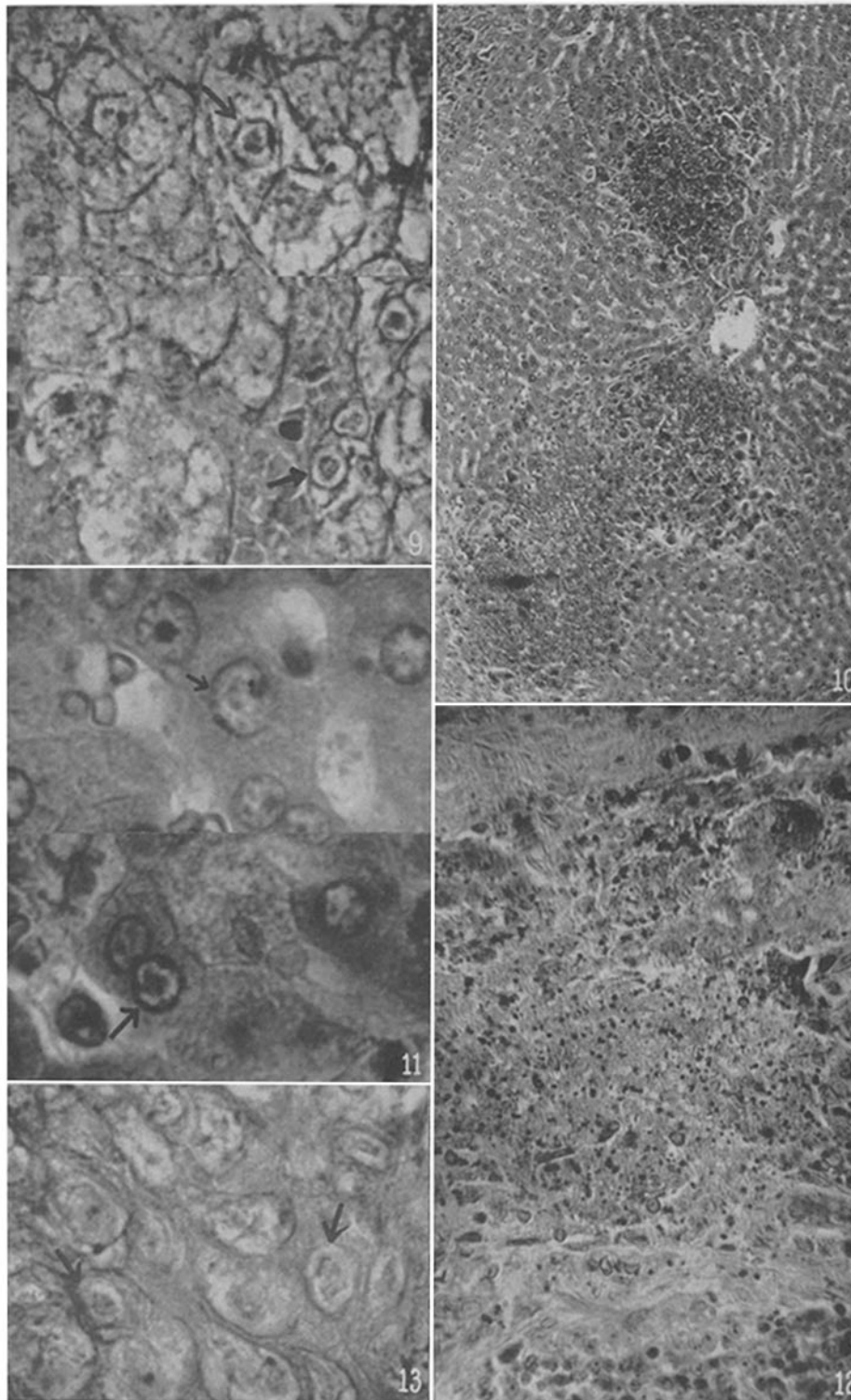
FIG. 13. Section of rabbit skin at site of inoculation with B virus; Zenker fixation and Giemsa stain. Almost all the epithelial cells in this zone contain eosinophilic intranuclear inclusions. $\times 1,210$.



(Sabin and Wright: Isolation of virus reproducing myelitis)



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